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Mitochondrial Aspartate Aminotransferase[1]) is a normal Constituent of human Serum

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There is no general agreement as to whether mitochondrial aspartate aminotransferase is a normal constituent of human serum, or not. This isoenzyme was studied in the serum of normal subjects by means of a new, quantitative method, involving anion-exchange column chromatography.
The normal serum contains about 2.65 mU/ml of mitochondrial isoenzyme.
This datum is compared with those of other Authors.

Die Anwesenheit der mitochondrialer Aspartat-Transaminase im Serum gesunder Personen, nicht von allen akzeptiert, wurde mit einer neuen quantitativen Methoden studiert, wobei die Chromatographie am Anionenaustauscher verwendet wurde.
Das Serum enthält ungefähr 2,65 mU/ml mitochondriales Isoenzym.
Diese Angabe wird mit jenen anderer Autoren verglichen.

There is no general agreement on the presence of mitochondrial aspartate aminotransferase in normal human serum. In fact, some authors found mitochondrial aspartate aminotransferase activity in the serum of normal subjects (1—4), while other workers were unable to detect this enzyme (5—11).
In order to clarify this problem, the mitochondrial iso enzyme of aspartate aminotranferase was studied in the serum of normal individuals by means of a very simple and specific quantitative method.

Material and Methods

Anion exchange column chromatography was used to separate mitochondrial aspartate aminotransferase. Columns were 0.9 cm in diameter and 15 cm in length. The ion-exchanger (DEAE Sephadex A 50 medium) was poured into the column, at room temperature, to obtain a final height of the settled suspension of 7—8 cm. The fresh sera, free from haemolysis, were dialyzed for 4 hours at 4°C in a continuous-flow apparatus, against 25 l of Na-phosphate buffer 0.008M, pH 7.
After dialysis, 1 ml of the sample was applied to the column at room temperature. After the sample had soaked into the column, elution was performed with 15 ml of Na-phosphate buffer 0.008M, pH 7.
The cytoplasmic isoenzyme is adsorbed by anion exchangers, such as DEAE Sephadex, while the mitochondrial component is not adsorbed (11). Therefore, only the mitochondrial aspartate aminotransferase is contained in the eluate.
If necessary, cytoplasmic aspartate aminotransferase activity can be either calculated by subtracting the mitochondrial activity from total activity of the dialyzed sample, or eluted with 15 ml of Na-phosphate buffer 0.2M, pH 7 + NaCl 0.2M.
Aspartate aminotransferase activity was assayed in serum before and after dialysis and in the eluates by the MonoTest (Biochemia-Boehringer & Co.).
Enzyme activities are expressed as mU/ml of serum.
Further details on this method, together with the results of experiments carried out to test its sensitivity, selectivity and reproducibility have been reported elsewhere (12). The sensitivity of the method has been now further increased by using the MonoTest for enzyme assays.

[1]) EC 2.6.1.1

Results

Twenty-six healthy individuals (18 males, 8 females), aged 18 to 49, were studied.
The mean mitochondrial aspartate aminotransferase values (± S. E.) in normal human serum are reported in Table 1. The values ranged from 1.10 mU/ml to 4.90 mU/ml. No differences were found between men and women. Mitochondrial aspartate aminotransferase constituted 25.77 ± 1.68% of the total aspartate aminotransferase activity.

Tab. 1
Serum levels of mitochondrial aspartate aminotransferase (AAT II) in normal subjects

	AAT II (mU/ml)	AAT II (% of total AAT)
No. of cases	26	26
$\bar{x}$	2.65	25.77
S. E.	± 0.19	± 1.86

Discussion

Mitochondrial aspartate aminotransferase is a normal constituent of human serum.
This finding is certainly not due to contamination by the cytoplasmic isoenzyme, since previous experiments have shown that no cytoplasmic aspartate aminotransferase activity was found in the first eluate, even when as much as 450 mU of this isoenzyme were applied to the column (12).
The negative findings reported by other Authors (5—11) were probably due to insufficient sensitivity of the methods they used, or to a loss of mitochondrial as-

partate aminotransferase activity, which is known to be very labile (11, 13).

Our data are in good agreement with those reported by BOYDE (3); however, BOYDE's semiquantitative method does not permit the detection of such small variations about the norm as does our quantitative method.

The values reported by BLOCK and COLL (1) and ROTZSCH and WENZEL (2) are so high as to cast doubt on the specificity of the electrophoretic procedure they used.

GABRIELI and ORFANOS (4), by means of a chromatographic technique similar to ours, found two aspartate aminotransferase fractions which were not adsorbed by the anion-exchanger. They speculate that the first eluted peak was the hepatic mitochondrial aspartate aminotransferase. The origin of the second cationic peak is not clear.

However, these authors should prove that they are really dealing with a separate isoenzyme, and not with contamination from cytoplasmic aspartate aminotransferase, since they did not dialyze their samples. It is well known in fact that the electrolytes which are present in serum may interfere with the polar groups of the ion-exchangers, and compromise the sharpness of the separation.

On the other hand, only one peak of non-adsorbed aspartate aminotransferase was found in dialyzed sera which were chromatographed in our laboratory by the technique described by GABRIELI and ORFANOS.

If the first eluted aspartate aminotransferase fraction only is considered as mitochondrial, the values found by GABRIELI and ORFANOS in the serum of normal subjects are lower than those observed in this study. This might be accounted for by the less sensitive colorimetric procedure that was used by these authors for the enzyme assay.

However, since GABRIELI and ORFANOS do not give any information either about the recovery of aspartate aminotransferase activity in the eluates, or about how they calculate the per cent isoenzyme activity, an exact evaluation of their data is not possible.

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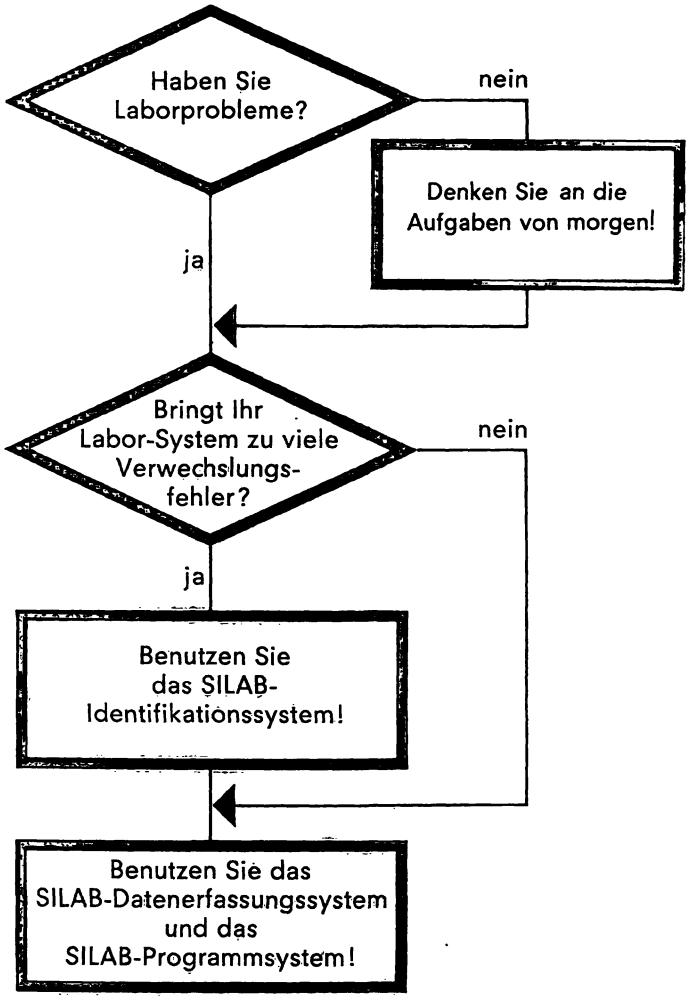

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